1-19-00

DATA EVALUATION RECORD

PROHEXADIONE-CALCIUM

Study Type: §82-7, Subchronic Oral Dietary Neurotoxicity Study in Rats

Work Assignment No. 1-02-25G (MRID 44457753)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer
Kimberly Woodard, B.S.

Signature: Mary Menetrez for Kimberly Woodard
Date: 7/26/99

Secondary Reviewer
Jay Early, M.S.

Signature: Jay Early
Date: 7/26/99

Program Manager
Mary L. Menetrez, Ph.D.

Signature: Mary L. Menetrez
Date: 07/26/99

Quality Assurance
Steve Brecher, Ph.D.

Signature: Steven Brecher
Date: 7/27/99

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

302

EPA Reviewer: Albin B. Kocialski Ph.D
Reregistration Action Branch I (7509C)
EPA Work Assignment Manager: Sanjivani Diwan, Ph.D.
Toxicology Branch I (7509C)

Albin B. Kocialski
Marion Copley 4/19/00

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Neurotoxicity [Feeding] - rat
OPPTS Number: 870.6200 OPP Guideline Number: §82-7

DP BARCODE: D246707 SUBMISSION CODE: S543930
P.C. CODE: 112600 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Prohexadione-calcium (92% a.i.)

SYNONYMS: Calcium salt of 3,5-dioxo-4-propionyl-cyclohexane-1-carboxylic acid

CITATION: Mellert, W., K., Kaufmann, W., and Hildebrand, B. (1996) Prohexadione-calcium, subchronic (90-day) oral dietary neurotoxicity study in Wistar rats. Department of Toxicology of BASF Aktiengesellschaft, Ludwigshafen/Rhein, FRG. Laboratory Project Identification Number 50C0277/94016. August 26, 1996. MRID 44457753. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, NC

EXECUTIVE SUMMARY: In a subchronic oral neurotoxicity study (MRID 44457753), prohexadione-calcium, (92% a.i.) was administered for 90 days to 10 Wistar Chbb:THOM (SPF) rats/sex/dose at dietary concentrations of 0, 1,000, 4,000, or 16,000 ppm (equivalent to 0/0, 70/85, 285/330, or 1148/1348 mg/kg/day [M/F]). Five animals/sex/group were perfused for neurohistological examination.

No animals died during the study. No treatment-related clinical signs, gross lesions, neoplastic tissue, or non-neoplastic tissue were observed. No changes were observed in food consumption, food efficiency, body weight, and body weight gain of treated animals when compared to concurrent controls.

There were no treatment-related findings during the homecage, open field, sensorimotor, and quantitative observations of the FOB tests indicative of neurotoxicity nor were there treatment-related changes in motor activity parameters in this study.

1
2

301

No treatment-related effects were seen in the mean absolute brain weights of the animals selected for perfusion fixation. No treatment-related neuropathological change was observed in any of the treatment groups.

The study LOAEL was not observed. The study NOAEL is ≥16,000 ppm (equivalent to 1148/1348 mg/kg/day [M/F], >1,000 mg/kg/day limit dose).

This 90-day neurotoxicity study is classified **acceptable (§82-7)** and satisfies the guideline requirements for a subchronic oral neurotoxicity study in rats.

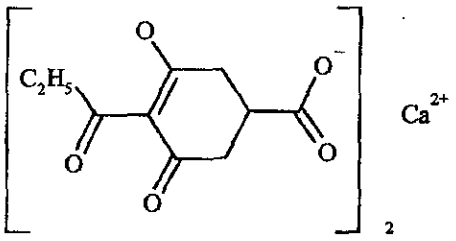
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

COMMENTS: The motor activity data are included in the Appendix.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Prohexadione-calcium
   Description: Solid, ivory colored powder
   Lot/Batch #: G14-37-114
   Purity: 92% a.i.
   Stability of compound: The compound was stable in the diet for 32 days when stored at room temperature.
   CAS #: 127277-53-6
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Rat
   Strain: Wistar (Chbb: THOM [SPF])
   Age and weight at the start of dosing: 49 days old; 208-247 g (males), 151-187 g (females)
   Source: Dr. Karl Thomae, GmbH, Biberach/Riss, FRG
   Housing: Individually in type DK III stainless steel wire cages with waste trays and bedding. Motor activity measurements were conducted in polycarbonate cages with wire covers.
   Diet: Kliba rats/mice/hamsters maintenance diet (343 meal, Klingentalmuhle AG, Kaiseraugst, Switzerland), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 20-24° C
   Humidity: 30-70%
   Air changes: Not reported
   Photoperiod: 12 hours light/12 hours dark
   Acclimation period: 14-17 days

B. STUDY DESIGN

1. In life dates - start: 1/30/95 (males) 2/1/95 (females)

end: 5/3/95 (males) 5/8/95 (females)

2. Animal assignment - The rats (10/sex/group) were randomly assigned to the test groups shown in Table 1 (stratified by weight) using a computerized random selection.

Table 1. Study design.[a]

Test Group	Concentration in the Diet (ppm)	Achieved Mean Dose (mg/kg/day)[b] [M/F]	Animals Assigned	
			Male	Female
Control	0	0	10	10
Low	1,000	70/85	10	10
Mid	4,000	285/330	10	10
High	16,000	1148/1348	10	10

a Dose selection was based on two previous studies performed on Fischer 344 rats. In a 4-week dietary study, rats (10/sex/dose) were fed prohexadione-calcium at dietary concentrations of 0, 300, 1,000, 3000, or 10,000 ppm. There were no signs of toxicity or treatment-related effects at any of the treatment levels; therefore, the study NOAEL was 10,000 ppm. In a 3-month feeding study, rats (10/sex/dose) were fed prohexadione-calcium at dietary concentrations of 0, 300, 1,000, 10,000, 30,000, or 50,000 ppm. There were no signs of toxicity or mortalities in any of the treatment groups. Food consumption was increased in rats fed at 30, 000 and 50,000 ppm without a concomitant increase in body weight. This effect was statistically significant ($p<0.01$) in males dosed at 50,000, but was deemed to be of no toxicological relevance. In addition, a dose-dependent increase in hyperplasia of squamous epithelium cells in the forestomach were observed in rats dosed at 10,000-50,000 ppm. The NOAEL was determined to be 1,000 ppm based on these findings.

b Mean daily test substance intake of 10 rats/sex/dose over 91 days, page 38 of the report.

c The limit dose is 1,000 mg/kg/day for subchronic neurotoxicity studies (870.6200).

3. Treatment preparation and dosing - The test substance was weighed, mixed with a small amount of food, and diluted to obtain desired concentrations. Preparation frequency was not reported.

To confirm the dietary concentrations of prohexadione-calcium, samples of prepared diet from each dose level were collected at weeks 1 and 8 of administration. Homogeneity was confirmed using triplicate samples of the 1,000 and 16,000 ppm dietary levels collected at the beginning of the study. Stability of the test substance in the prepared diet was determined after the in-life phase of the study. Prohexadione-calcium was mixed with the diet to a concentration of approximately 60 ppm and stored at ambient temperature; samples were collected 32 days after mixing and analyzed.

Results:

Concentration analysis (% of nominal):

1,000 mg/kg: 95-98.9%

4,000 mg/kg: 97.8 and 104.5%

304

16,000 mg/kg: 95.4-98.4%

Homogeneity (% nominal ± coefficient of variation):
1,000 mg/kg: 96.1-98.9 ± 1.4%
16,000 mg/kg: 95.4-98.4 ± 1.75%

Stability (storage at 25° C):
32 days: 93.0-101.3% of nominal

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics - The mean with standard deviation was presented for each parameter. For data on rearing, motor activity counts, grip strength and landing foot-spread tests, feces, and brain weights, a non-parametric one way analysis of variance (ANOVA) was performed using the Kruskal-Wallis-H test. If differences were significant at $p<0.05$, then a Mann-Whitney-U test or the Wilcoxon test (brain weights) was conducted for comparison with the control group. For data on food consumption, body weight, body weight change, and food efficiency, a parametric one-way ANOVA was done using the F-test. If significant differences ($p<0.05$) were indicated by the ANOVA, then a Dunnett's test was used to compare control and treated groups.

C. METHODS.

1. Observations

A. Clinical signs - Animals were examined twice daily on weekdays and once daily on Saturdays, Sundays, and holidays for an overall state of health. Detailed observations and palpations took place once weekly.

B. Functional observational battery (FOB) and motor activity - All animals were subjected to an FOB and motor activity measurement on Days -7 (acclimation), 22, 50, and 85. The FOB assessment included the following parameters:

Home cage observations
Posture
Tremors/Convulsions
Behavior
General Observations
Gait
Abnormal movements

Sensorimotor/Reflex observations
Startle response
Visual placing response
Tail pinch response
Olfaction
Pupillary reflex
Winking reflex
Pinna reflex
Olfaction
Descending from box (catalepsy)
Righting response
Behavior during handling
Vocalization

Open field observations
Behavior when removed from cage
Activity/Arousal level
Posture
Gait
Bizarre behavior
Rearing count
Salivation
Respiration
Lacrimation
Exophthalmus
Fur appearance
Skin color
Tremors/Convulsions
Abnormal movements
Palpebral closure
Feces/Urination

Neuromuscular tests (Quantitative)
Hindlimb grip strength
Forelimb grip strength
Landing foot spread

Following each administration of the FOB, motor activity was assessed using a Multi-Varimex-System (Columbus Instruments Int. Corp., Inc., OH) with 4 infrared beams per cage to measure total activity (counts). Motor activity was evaluated over a 90 minute period and the number of beam interruptions was tabulated for 18 intervals, each lasting 5 minutes.

C. Positive controls - Summaries were provided of six neurotoxicity studies performed on Wistar rats to generate positive control data and validate the laboratory's procedures and the ability of the observers to perform the FOB and assess motor activity, neurotoxicity and behavioral effects. The chemicals used in these studies included: acrylamide (0 or 40 mg/kg, administered 5 days/week for 2 weeks by gavage); trimethyltin chloride (0, 6, 9, or 12 mg/kg, administered as a single i.p. injection); 3,3'-iminodipropionitrile (IDPN, 0 or 2 g/kg administered as a single i.p. injection); carbaryl (0, 10, or 30 mg/kg, administered as a single i.p. injection); nomifensin (0 or 10 mg/kg, administered as a single oral dose); and diazepam (0 or 3 mg/kg, administered as a single oral dose).

Clinical signs of peripheral neuropathy such as ataxia and/or limb weakness were observed after administration of acrylamide, trimethyltin chloride, carbaryl, and IDPN. Signs of central neuropathy, such as tremors and convulsions, were also observed in animals treated with trimethyltin chloride and carbaryl. Signs of autonomic system involvement, such as salivation, were observed in the animals treated with IDPN and carbaryl. Histopathological evidence of peripheral and central nervous system changes were noted following administration of acrylamide, trimethyltin chloride, and IDPN. Increased motor activity was observed in the nomifensin treated animals and decreased activity was observed in the diazepam treated animals.

2. Body weight - Animals were weighed prior to randomization, at the start of treatment (day 0), at weekly intervals during the study, and each time the FOB was performed. Body weight changes were reported as the change in weight from day 0 to day_n.

3. Food and compound intake/food efficiency - Food consumption for each animal was measured at weekly intervals throughout the treatment period and was reported as g/animal/day. Mean compound intake was calculated as mg/kg/day. Food efficiency (group means) was also calculated based on weekly changes in body weight and food consumption.

4. Sacrifice and pathology - At study termination, 5 rats/sex/dose were anesthetized and perfused *in situ* and the remaining animals were sacrificed by CO_2 inhalation. Perfused animals were necropsied and organs were examined for gross pathology. Central and peripheral nervous system tissues were dissected and preserved. Absolute brain (without olfactory bulb) weights were recorded after removal, and any abnormalities in the brain or spinal cord were noted. Nervous system tissues from the 1,000 and 4,000 ppm dose groups were not examined, but were fixed and stored in buffered solutions. For control and 16,000 ppm dosed rats, central nervous system tissues (excluding the dorsal and lumbar ganglions and fibers) and the gastrocnemius muscle were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Dorsal and lumbar roots ganglions and fibers and peripheral nervous system tissues were embedded in plastic, sectioned, and stained with Azure II-Methylene blue- basic Fuschin (AMbF). For neuropathological examination, the following tissues from 5 rats/sex from the control and high-dose groups were examined qualitatively:

Central Nervous System		
Brain		
Frontal lobe	Parietal lobe	Midbrain
Pons	Cerebellum	Medulla oblongata
Spinal cord		
Cervical swellings C_3-C_6	Lumbar swellings L_1-L_4	
Other		
Gasserian ganglion w/ nerve	Cervical dorsal root ganglion at C_3-C_6	Cervical dorsal root fibers at C_3-C_6
Cervical ventral root fibers at C_3-C_6	Lumbar dorsal root ganglion at L_1-L_4	Lumbar dorsal root fibers at L_1-L_4
Lumbar ventral root fibers at L_1-L_4		
Peripheral Nervous System		
Gastrocnemius muscle	Proximal sciatic nerve	Sural nerve
Tibial nerve		

II. RESULTS

A. Observations

1. Mortality - No animals died during the study.

2. Clinical signs - No treatment-related changes were observed in appearance or behavior of the rats during the study.

3. Functional observational battery - Results of the FOB indicated no treatment-related findings during the home cage and open field observations. During the sensorimotor observations, 1/10 high-dose females exhibited a resistance to handling on days 50 and 85. During the catalepsy test on day 50, one control male and one mid-dose female left the given position slowly and on day 85 one mid-dose male and two high-dose males left the position slowly. These findings were sporadic and not dose related and therefore considered to be incidental and not treatment related.

4. Motor activity - During each 90-minute assessment period, motor activity in all animals including controls declined over time. However, in male rats dosed with prohexadione-calcium, there was what appeared to be a trend toward decreased activity at earlier intervals relative to the control group. Compared to control males, activity was decreased (not statistically significant) on day 22 in low- (↓15-88%), mid- (↓51-97%), and high-dose (↓71-99%) males during intervals 11-15. On day 50 (intervals 5-18), activity was again decreased in low (↓32-86%), mid (↓18-91%), and high-dose males (↓19-93%). However, the effect was less pronounced by day 85, with decreases occurring during interval 7-15 (low - ↓8-70%; mid - ↓8-87%; high - ↓27-68%). Although there was a general trend toward decreased motor activity in males, significant ($p \leq 0.05$ or 0.01) decreases in motor activity in males occurred only in the mid- and high-dose males on day 50 during interval 15 (↓91% and ↓54%, respectively).

   Significant ($p \leq 0.05$ or 0.01) decreases in motor activity were observed for females on day 50 during interval 2 in the low- (↓22%), mid- (↓33%), and high-dose (↓23%) groups and on day 85 during interval 18 in low-dose females (↓96%) and during intervals 10 and 11 for high-dose females (↓96-98%). However, an overall trend toward decreased activity was not evident in females.

   The toxicological significance of the decreased motor activity observed in treated males is equivocal given that the decreases were only significant in a couple of instances, the decreases were transient, and a similar trend in decreased motor activity was not observed in treated females.

B. Body weight and body weight gain

No treatment-related changes in body weight and body weight gains were observed in any of the treatment groups.

C. Food and compound intake/water consumption

1. Food consumption - Food consumption and food efficiency in all treatment groups was similar to the concurrent controls.

2. Compound consumption - The achieved mean dosages based on nominal dietary concentrations, actual body weights, and actual food consumption are shown in Table 1.

D. Sacrifice and pathology

1. Organ weight - No treatment-related effects were seen in the mean absolute brain weights of the animals selected for perfusion fixation.

2. Gross pathology - No treatment-related gross postmortem findings were observed in any of the treatment groups including the animals selected for perfusion.

3. Microscopic pathology

a) Non-neoplastic - No treatment-related neuropathological change was observed in any of the groups treated with prohexadione-calcium. However, moderate dilation of the lateral ventricles of the brain was observed in a female control and minimal axonal degeneration was observed in the tibial nerve of one male control and the sciatic nerve of a female control.

b) Neoplastic - No neoplastic tissue was observed in the treated or control rats.

III. DISCUSSION

A. Investigator's conclusions

The study author concluded that prohexadione-calcium at dietary concentrations of ≤16,000 ppm does not elicit a toxic effect. No treatment-related signs of neurotoxicity or neuropathological changes were noted throughout the study. A NOAEL of 16,000 ppm was observed.

B. Reviewer's discussion

In this subchronic neurotoxicity study, prohexadione-calcium was administered for 90 days to 10 Wistar rats/sex/dose at dietary concentrations of 0, 1,000, 4,000, or 16,000 ppm (equivalent to mean achieved doses of 0/0, 70/85, 285/330, or 1148/1348 mg/kg/day [M/F]).

No animals died during the study. No treatment-related clinical signs, gross lesions, neoplastic tissue, or non-neoplastic tissue were observed. No changes were observed in food consumption and efficiency, body weight, body weight gain, and absolute brain weight of treated animals when compared to concurrent controls.

There were no treatment-related findings during the homecage, open field, sensorimotor, and quantitative observations of the FOB tests indicative of neurotoxicity in this study. No treatment-related neuropathological changes was observed in any of the treated groups.

Although there was a trend toward decreased motor activity in males, the toxicological significance of this effect is equivocal as the decreases were only significant in a couple of instances and were transient in nature. In addition, a similar trend in decreased motor activity was not observed in treated females.

The study LOAEL was not observed. The study NOAEL is ≥16,000 ppm (equivalent to 1148/1348 mg/kg/day [M/F/], >limit dose).

This 90-day subchronic neurotoxicity study is classified **acceptable (§82-7)** and satisfies the guideline requirements for a neurotoxicity study in rats.

IV. STUDY DEFICIENCIES

The study was conducted according to Subdivision F guidelines and contained no deficiencies and/or deviations that would affect the study results.

DER - Subchronic Oral Neurotoxicity Study Rats
MRID 44457753

Page____ is not included in this copy.

Pages 12 through 30 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
